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Root Morphological Responses of Oil Palm (*Elaeis guineensis* Jacq.) Hybrids to Copper Toxicity

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ABSTRACT

The experiment aimed to identify the root response of eight oil palm hybrids to copper toxicity. The factorial treatments were arranged in Randomized Completely Block Design with three blocks as replication. The first factor was the copper toxicity, while eight oil palm hybrids (DxP) consisted of Yangabi (P1), Avros (P2), Langkat (P3), PPKS 239 (P4), Simalungun (P5), PPKS 718 (P6), PPKS 540 (P7), and Dumpy (P8) as second factor. Root growth variables were observed, including total root length, total root area, root volume and diameter, copper content on root, fractal dimension, relative root water content, fresh root weight, and root dry weight. Data were analysed using analysis of variance (ANOVA) and continued with Duncan's Multiple Range Test at $\alpha=5\%$. There was a declining in total root length, volume and diameter, fresh and dry weight as the copper content rose on the root tissue, but no significant different was found in total root area.

Keywords: Copper Toxicity, Oil Palm, Root

INTRODUCTION

Copper (Cu) is a micro essential for plant. Cu has a vital function in electron transport in photosynthesis, as well in respiration and as a cofactor in various enzymies, such as superoxide dismutase, cytochrome c, oxidase, and plastocyanin (Clemens, 2001). Cu is mostly found in chloroplast (>50%) and is bound to plastocyanin (Marschner, 1995). Cu has a high affinity to dioxygen molecules, which explains how Cu is a metal catalyst of many oxidation reactions. This main group member is mitochondria cytochrome oxidase as the main catalyst of terminal oxidation. Cu is also found at electron protein carrier such as plastocyanin (Pilon *et al.*, 2006).

Naturally, Cu is in the range 2 to 100 ppm in soil with the average of about 30 ppm. In many plants, Cu content is about 8 - 20 ppm. Without an adequate Cu supply, plants growth will decline. Otherwise, an excessive amount of Cu in soil can cause toxicity on plant. The sources of soil toxicity are from human activity, such as mining, industrial waste, agriculture (fungicide, etc) (Gardner *et al.*, 1985; Mito *et al.*, 2014). Excessive amount of Cu can cause structural anatomical, morphological, and physiological

changes, such as damaging root tissue, declining photosynthetic rate and growth (Lequeux *et al.*, 2010). The high concentration of Cu will first inhibit root growth, then shoot growth. This is not because root is more sensitive in high Cu concentration, but plant localised copper in root and inhibit the translocation to the shoot (Broadley *et al.*, 2012).

In high Cu level, 60% total Cu in root can be bound to cell wall and plasma membrane of cell wall fraction. Additionally, Cu mobility process in root or Cu absorption reduction through extracellular bond with rooting exudate in some Cu tolerance mechanisms (Yruela, 2009).

In the vine (*Vitis labrusca* L.) seedling, root planted in the soil with excessive Cu without liming shows obvious toxicity symptoms, such as shortening of the differential root cell area, declining size of root apex, larger root diameter, cortex area, cortical cell area, and silindrical area of vascular tissue, then the increasing of total cell that has phenolic content (Ambrosini *et al.*, 2015).

Cu toxicity symptoms shown in some of root area among others are on apex area, the changing structure of wall cell and tissues, root shortening and thickening,

increasing of total lateral root, and plasmolysis on epidermis cell which can decreasing the root density (Chen *et al.*, 2013; Juang *et al.*, 2014). This experiment aimed to figure out oil palm root response from eight different hybrids (DxP) Yangabi (P1), Avros (P2), Langkat (P3), PPKS 239 (P4), Simalungun (P5), PPKS 718 (P6), PPKS 540 (P7), and Dumpy (P8) to copper toxicity

MATERIALS AND METHODS

This experiment had been conducted on November 2014 to Mei 2015 at Bendosari, Madurejo, Prambanan, Sleman and Plant Science Laboratory, Agriculture Faculty of Gadjah Mada University, Yogyakarta.

The experiment held at main nursery level (9 months after planting) from November 2014 to Mei 2015, within 8 different oil palm hybrids (Yangabi (P1), Avros (P2), Langkat (P3), PPKS 239 (P4) Simalungun (P5), PPKS 718 (P6), PPKS 540 (P7) and Dumpy (P8)) and with treatment of toxicity (first with 300 ppm Cu toxicity from $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ by irrigation and with no copper adding as a control treatment). The application of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was given from the 37th weeks after replanting on main nursery stage. On the 38th and 48th week after replanting on the main nursery stage, sample plants were taken to the laboratory to measure the total root length and area, root diameter and volume, fractal dimension, relative root water content, Cu content, root and shoot biomass, plant height, total leaves, root response to Cu toxicity. Data were analysed using analysis of

variance (ANOVA) and followed with Duncan's Multiple Range Test at 5% alpha.

RESULT AND DISCUSSION

The additions of Cu to the plant media significantly increased the Cu content and absorption on root of *Elaeis guineensis* Jacq. at main nursery stages (Table 1). In control plants, the Cu content and absorption were still in normal level. There was not any significant difference among eight *Elaeis guineensis* Jacq. hybrids. According to Mocquot *et al.* (1996), Cu toxic critical value in plant was 26 mg Cu per dry weight on root and 21 mg Cu per dry weight on leaf. In this research, Cu in root with Cu treatment was up to 18.86 mg per root dry weight, which was still in the range under critical value on plant, eventhough the concentration of Cu on root was much higher in plant with Cu treatment than control.

The increasing Cu level on root tissues caused by the adding of 300 ppm Cu on the media caused some structural damages to the root tissue as the ROS activity increased. Excessive Cu in root tissue could generate reactive oxygen species (ROS), including O_2^- , H_2O_2 , and the HO^\cdot radical, which caused lipid peroxidation and might caused membrane permeability damage (Andrade *et al.*, 2010) led to ion leakage (De Vos *et al.*, 1991), and also disrupt the activity of membrane-bound enzymes (Del Rio *et al.*, 2006). Cell membrane was the primary site of Cu toxicity as proven by the increased lipid peroxidation (Fidalgo *et al.*, 2013).

Table 1. Cu content and absorption on oil palm (*Elaeis guineensis* Jacq.) root at 48 week after replanting at main nursery stage

Treatment	Cu Content on Root Tissue (ppm)	Cu Absorption on Root (mg/Root Dry Weight)
Cu Treatment (ppm)		
0	23.41 b	0.69 b
300	1395.08 a	18.86 a
Oil Palm Hybrids		
Yangambi	991.28 a	14.34 a
Avros	538.96 a	8.55 a
Langkat	710.62 a	10.15 a
PPKS 239	733.97 a	8.65 a
Simalungun	822.58 a	9.64 a
PPKS 718	457.90 a	4.50 a
PPKS 540	530.10 a	7.22 a
Dumpy	888.55 a	15.14 a
Interaction	(-)	(-)
CV (%)	25.62	16.09

Remarks: The means in one column followed by the same letter were not significantly different according to DMRT (α 5%). (-) There is no interaction between the factors tested.

Table 2. Relative root water content on 38 and 48 week after replanting on main nursery stage

Treatment	Relative Root Water Content (%)	
	38	48
Cu Treatment (ppm)		
0	36.89 a	46.00 a
300	38.36 a	32.42 b
Oil Palm Hybrids		
Yangambi	47.69 a	37.98 a
Avros	31.93 a	41.09 a
Langkat	42.38 a	44.34 a
PPKS 239	40.39 a	36.24 a
Simalungun	36.90 a	34.62 a
PPKS 718	40.30 a	37.24 a
PPKS 540	38.17 a	39.04 a
Dumpy	23.28 a	43.11 a
Interaction	(-)	(-)
CV (%)	24.67	39.37

Remarks: The means in one column followed by the same letter were not significantly different according to DMRT (α 5%). (-) There is no interaction between the factors tested.

Table 3. Responses of the oil palm hybrids to Cu toxicity on root growth at 48 weeks after replanting

Oil Palm Hybrids	Δ Responses on Root Growth (%)				
	Root Length	Root Area	Root Diameter	Root Volume	Fractal Dimension
Yangambi	40.23 a	35.20 a	1.64 ab	34.74 a	-7.22 a
Avros	-908.45 a	-192.20 a	41.43 a	44.43 a	-0.53 a
Langkat	39.11 a	44.66 a	10.12 ab	45.56 a	-2.10 a
PPKS 239	48.15 a	54.11 a	5.21 ab	58.00 a	-2.38 a
Simalungun	-7.56 a	25.72 a	19.01 ab	17.85 a	-2.67 a
PPKS 718	-76.09 a	-22.45 a	18.36 ab	45.27 a	-4.46 a
PPKS 540	-26.03 a	7.04 a	19.94 ab	27.90 a	-1.62 a
Dumpy	27.56 a	12.05 a	-25.25 b	36.36 a	-0.86 a
Average	-107.885	-4.84	11.31	38.78	51.37
CV(%)	24.429	29.828	18.933	27.933	15.011

Remarks: The means in one column followed by the same letter were not significantly different according to DMRT (α 5%).

Cu toxicity might also result from the strong interactions of Cu with sulfhydryl groups of enzymes and protein in the apoplast of root cell, which could inhibit enzyme activities or caused changes in the structure and replacement of key elements, resulting in deficiency of other nutrients (Yruela, 2005; Kabala *et al.*, 2008). This could affect nutritional status of the plant. In some studies, excessive Cu could lead to Fe deficiency (Marschner, 1995). It could also lead to nutritional imbalanced which inhibited plant growth and biomass production. It was obviously seen from the declining of shoot and root, both in fresh and dry biomass.

Relative root water content decreased by the Cu

stress treatment at 48 weeks after replanting on main nursery stage observation, but there was no significantly difference in oil palm hybrids (Table 2). In excess of Cu, root tissue lowered its availability of root permeability as a mechanism to inhibit Cu ion entered the root tissue by lignification in the membrane cell of some tissues. In fact, Cu was one of the cofactors in lignin synthesise. The increasing of Cu level in root tissues was followed by the rising of lignification.

Lignin, comprising of phenolic hetero polymers, was a complex component of cell wall (Lin *et al.*, 2005). The adding of nano-CuO on *Glycine max* at 100, 200, 400, and 500 mg/L enhanced the lignification of root cells, thereby affecting the root development

Table 2. Relative root water content on 38 and 48 week after replanting on main nursery stage

Treatment	Relative Root Water Content (%)	
	38	48
Cu Treatment (ppm)		
0	36.89 a	46.00 a
300	38.36 a	32.42 b
Oil Palm Hybrids		
Yangambi	47.69 a	37.98 a
Avros	31.93 a	41.09 a
Langkat	42.38 a	44.34 a
PPKS 239	40.39 a	36.24 a
Simalungun	36.90 a	34.62 a
PPKS 718	40.30 a	37.24 a
PPKS 540	38.17 a	39.04 a
Dumpy	23.28 a	43.11 a
Interaction	(-)	(-)
CV (%)	24.67	39.37

Remarks: The means in one column followed by the same letter were not significantly different according to DMRT (α 5%). (-) There is no interaction between the factors tested.

Table 5. Plant responses to Cu toxicity on shoot growth (%)

Oil Palm Hybrids	Δ Responses on Shoot Growth (%)	
	Plant Height	Total Leaf
Yangambi	40.399 a	6.349 a
Avros	-3.370 b	6.435 a
Langkat	50.009 a	18.238 a
PPKS 239	53.899 a	7.773 a
Simalungun	39.632 a	12.081 a
PPKS 718	48.846 a	18.446 a
PPKS 540	51.574 a	16.173 a
Dumpy	46.826 a	13.127 a
Average	40.98	12.33
CV(%)	13.418	23.365

Remarks: The means in one column followed by the same letter were not significantly different according to DMRT (α 5%).

(Nair and Chung, 2014). Increasing lignification could happen because there were at least two Cu enzymes in lignin biosynthesis: polyphenol oxidase catalysed the oxidation of phenolics as precursor of lignin, and diamine oxidase provided the H_2O_2 required for oxidation by peroxidases (Broadley *et al.*, 2012).

The Δ responses of total root length, surface area, diameter, and volume of Yangabi (P1), Avros (P2), Langkat (P3), PPKS 239 (P4) Simalungun (P5), PPKS 718 (P6), PPKS 540 (P7), and Dumpy (P8) were shown on Table 3. The hybrids showed different response on root diameter, where Avros hybrid had a greater declining in root diameter. On the other hand, Dumpy hybrid seemed to develop a bigger diameter than other hybrids. Meanwhile, there was

no significant difference in root length, area, volume, and fractal dimension.

In excessive Cu, all oil palm hybrids developed their root formation pattern as their fractal dimension increased. Yangambi, Langkat, and PPKS 239 showed the same response in root growth inhibition with decreased root length, surface area, diameter and volume. Avros and PPKS 718 developed more on root length and surface area despite their decreased root diameter and volume. Simalungun and PPKS 540 developed root length despite suffered from inhibition on root surface area, diameter, and volume. Dumpy developed root diameter despite the inhibition of other root growth parameters.

According to Sheldon and Menzies (2004) research

on Cu toxicity effect on Rode grass as a bioaccumulator for ex-mining land rehabilitation, Cu stress led to an inhibition in root growth with several symptoms, such as deformation of root tissue, root cuticle damage, degradating root hair to root structural deformation. The degradating root growth showed at Cu concentration less than 1 μM with invincible damage at 0.2 μM .

Copper stress induced the synthesis of oxylipins through enzymatic mechanisms (Ritter *et al.*, 2008), which then led to root waving, loss of apical dominance, and a decrease in root elongation (Velloso *et al.*, 2007). These actions inhibited root cell growth, such as cell propagation and differentiation, then the accumulation of this reaction was decreasing the root area and length growth, with increased root diameter as a result of lignification in some cell as the increasing of Cu level. This was one of plant defense mechanism against Cu toxicity.

There was no significantly different responses on all parameters of biomass (Table 4). Plant with Cu stress significantly inhibited their biomass translocation to shoot and root for growth in all of palm oil hybrids in this research. In excessive Cu condition, *Zea mays* (Mocquot *et al.*, 1996) and *Lupinus termis* (Gadallah and El-Enany, 1999) also gave the same responses as declining metabolisms and physiological reaction on plant led to dry biomass degradation.

Cu was important in many physiological processes on plants, such as photosynthesis and respiration. The thylakoid membrane in the chloroplast, especially photosystem II (PSII) was a primary target of Cu toxicity (Bernal *et al.*, 2004). The elevating of ROS on thylakoid membrane led to an ultrastructural damage in chlorophyll and caused inhibition on photosynthesis by decreasing the chlorophyll content on leaf. The damage of membrane and chlorophyll in leaf was shown with necrotic area in leaf at high level of Cu toxicity, with yellowing interveinal chlorosis in the early stage of Cu toxicity. It led to a decline in biomass productions as the result of photosynthesis.

Declining in biomass production on plants as the photosynthesis decreased led to a declining in shoot growth. We could observe it from plant height and total leaves per plant which were reduced compared with the control treatment.

In almost all hybrids, Cu stress inhibited shoot growth, such as plant height and total leaves, except in Avros hybrids (Table 5). In excessive Cu condition, Avros was able to maintain its shoot growth, including

plant height and total leaves per plant. When others hybrids' shoot growth decreased in high Cu level toxicity, Avros had better shoot growth.

The inhibition of root growth had a bad impact to shoot growth. Root damage reduced its ability on absorption nutrition from media, thus the translocation from root to shoot was reduced as the Cu level on root increased. These had significantly affected all metabolisms and physiological reactions in shoot, such as photosynthesis and respiration. It was obviously seen in the decreasing of plant height and total leaves per plant of oil palm exposed to excessive Cu. Typically, plants which were given excessive Cu had an increased Cu content in their roots. Several studies reported that excessive Cu did not reach shoot, at least in the first days of Cu exposure (Navari-Izzo *et al.*, 2006).

CONCLUSION

Cu stress in oil palm inhibit the root growth, such as declining in root length, volume, and biomass. The declining in root growth, such as root length, area, diameter, volume, and fractal dimension, led to an inhibition in shoot growth, such as plant height, leaves, and plant biomass.

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